in the LGN of mice lacking C1q or C3 is remarkably similar to that seen in mice deficient for cell surface MHC class I (A. Datwani and C.J. Shatz, personal communication). Thus, it is possible that C1q and C3 act by inducing expression of MHC class I in the developing visual system. Future work might resolve this question by determining whether MHC class I levels are reduced in mice lacking C1q or C3 (or vice versa) and assessing whether the effects of MHC class I deficiency and C1q deficiency on visual system development occlude one another, which would suggest that they act in functionally convergent pathways.

Activated microglia are the only cells of the brain known to express C3 receptors (Gasque et al., 1998), indicating that they may participate in synaptic remodeling mediated by C3 (Figure 1). If microglia contribute to this process, one prediction is that higher numbers of microglia should be present in the developing brain specifically during the period of active remodeling. This is in fact the case in some brain regions (e.g., Maslinska et al., 1998). High numbers of activated microglia have also recently been reported in postmortem brain samples from patients with autism (Vargas et al., 2005), raising the possibility that microglia might contribute to pathological changes in connectivity in this neurodevelopmental disorder.

The results reported by Stevens et al. add to growing evidence that the immune system and nervous system make different use of some of the same molecular machinery. This molecular overlap could act as a point of either beneficial or harmful cross-talk between the two systems in injury and disease states and hints at new therapeutic directions for a wide variety of neurological disorders. Much additional work is needed to elucidate the precise molecular mechanisms by which proteins of the innate and adaptive immune system also participate in normal brain development and plasticity. Fortunately, this work will be greatly facilitated by the knowledge and experimental tools established when these proteins were first characterized in the immune system.

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The Exoneme Helps Malaria Parasites to Break out of Blood Cells

Chris J. Janse[1] and Andrew P. Waters[1,*]
[1]Department of Parasitology, Leiden University Medical Centre, Albinusdreef 2, 2333ZA Leiden, The Netherlands
*Correspondence: waters@lumc.nl
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Malaria parasites must invade the erythrocytes of its host, to be able to grow and multiply. Having depleted the host cell of its nutrients, the parasites break out to invade new erythrocytes. In this issue of *Cell*, Yeoh et al. (2007) discover a new organelle, the exoneme, that contains a protease SUB1, which helps the parasite to escape from old erythrocytes and invade new ones.

The malaria parasite (*Plasmodium*) is a unicellular, obligate intracellular protozoan that must invade, colonize, replicate within, and emerge from various cells types of the mammalian host or mosquito vector in order to complete its life cycle. Although the basic structure of *Plasmodium* is comparable to a standard eukaryotic cell, it is capable of producing distinct invasive forms and specialized organelles that are evolved to recognize and invade the correct cell type. For growth and multiplication in the bloodstream of its vertebrate host,

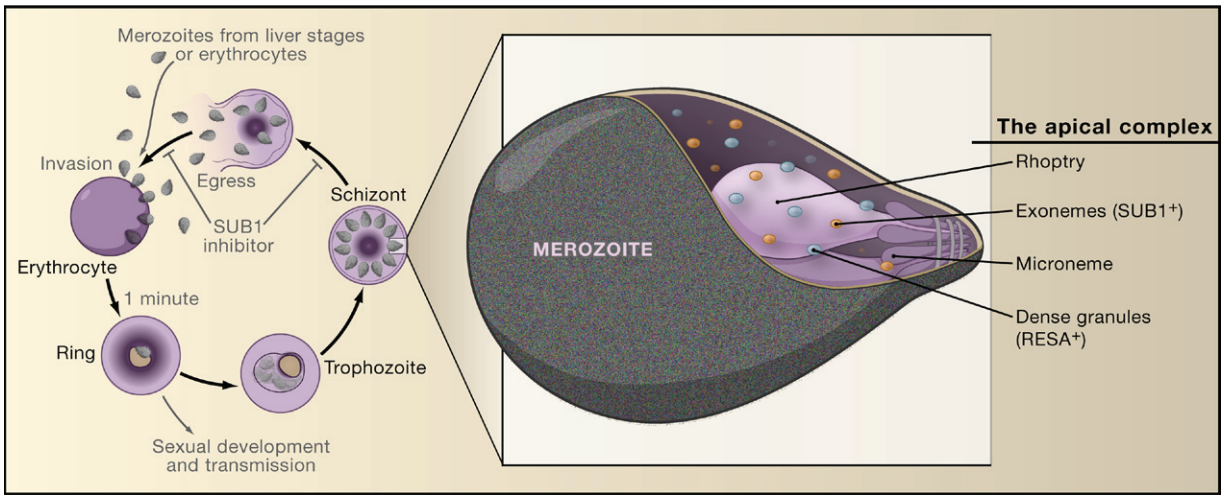

Figure 1. The Blood Stage in the Life Cycle of the Malaria Parasite

Propagation of malaria parasites in the blood begins with invasion of an erythrocyte by a merozoite from an infected liver cell or from a previously infected erythrocyte. For invasion, the merozoite uses three organelles: rhoptries, micronemes, and dense granules that are involved in merozoite attachment, reorientation, invasion, and formation of the vacuole in which the parasite grows (the parasitophorous vacuole). Now, Yeoh et al. (2007) reveal the presence of a new organelle, the exoneme, that is involved in the release of merozoites from the old erythrocyte. These organelles contain the protease SUB1 that play a key role in activating the host SERA proteases that are associated with destruction of the parasitophorous vacuole and the erythrocyte membrane. In addition to preventing egress of merozoites, an inhibitor of SUB1 also prevents merozoite invasion of the erythrocyte, indicating that SUB1 may also promote the maturation of malarial proteins that are involved in invasion.

the malaria parasite is dependent on erythrocytes, which it invades and inhabits for 24–72 hr, consuming hemoglobin and forming 8–32 "daughters" or merozoites (Figure 1). For further survival, these merozoites leave the old, depleted erythrocyte and find a new erythrocyte (Figure 1). Yeoh et al. (2007) provide important new insights into how merozoites make their escape from depleted erythrocytes.

The recognition and invasion of new erythrocytes by the malarial merozoite has been well-studied (reviewed in Crabb and Cowman, 2006). The invasive forms of this and other related parasites are defined by the presence of an apical complex of organelles—hence their grouping into the phylum Apicomplexa—that are associated with the invasion processes. Electron micrographs have revealed the fine ultrastructural detail of all invasive forms of *Plasmodium.* In merozoites, the apical complex contains three typical, distinct organelles identified by shape, protein content, and function: a pair of rhoptries, numerous micronemes, and approximately 50 dense granules (Figure 1). Although the functions of the three organelles overlap, in general rhoptries contain proteins essential for parasite recognition and invasion of the host cell—particularly in the merozoite invading the erythrocyte—and also lipid stocks that help establish the parasitophorous vacuole that the colonizing parasite must establish around itself (reviewed in Kats et al., 2006). Micronemes contain additional adhesins used to identify and attach to the target host cell and to link to actomyosin filaments for gliding and invasion (reviewed in Dowse and Soldati, 2004). Dense granules contain proteins that establish the lines of communication between the parasitophorous vacuole membrane and the host cell and beyond (reviewed in Mercier et al., 2005).

Blackman and colleagues (Yeoh et al., 2007) now demonstrate that merozoites have not only developed specific organelles for invasion of erythrocytes but also for egress from these cells. They show that the dense granules of the apical complex are more heterogeneous than previously recognized and that their protein content and function can be sufficiently distinct to identify new organelles within their midst. Welcome the exoneme to our lexicon. The identification of the exoneme stemmed from the group's long-standing interest in the role of proteases in merozoite egress from the infected erythrocyte and erythrocyte invasion. Studies with broad-range protease inhibitors had revealed the crucial nature of numerous (serine and papain-like) protease activities for several merozoite-associated events coupled to both erythrocyte invasion and merozoite release upon rupture of the infected erythrocyte (Delplace et al.,1988; Wickham et al., 2003). Focusing on merozoite release, it is in principle at least a two-step process as two membranes, the parasitophorous vacuole membrane and the infected erythrocyte membrane, must be ruptured in order to secure merozoite release. Of the three subtilisin-like serine proteases encoded in the conserved *Plasmodium* genome, one (termed SUB1) possessed an expression profile consistent with a role in parasite egress, that is it's expression peaks at schizont maturity (the schizont is the form of the parasite in infected erythrocytes that contains fully mature daughter merozoites). Blackman's group had previously characterized SUB1 from the lethal human parasite *Plasmodium falciparum* (PfSUB1) and demonstrated its distinctive structural properties that make it a promising target for drug

development (Withers-Martinez et al., 2002). In the current study, Yeoh et al. (2007) show that *pfsub1* is an essential gene. The authors engineered a C-terminal epitope HA-tagged version of PfSUB1 (PfSUB1HA3) to follow its localization. They noticed that PfSUB1HA3 localizes to an apical disposed structure that resembled a dense granule but did not colocalize with a marker of dense granules such as the ring-infected erythrocyte surface antigen RESA. Furthermore, although RESA and other proteins are found in newly infected erythrocytes, PfSUB1HA3 was not. In fact, PfSUB1HA3 could be found only in supernatants of ruptured schizont cultures, implying that the PfSUB1HA3 granule was released significantly earlier than the RESA-dense granule and immediately prior to rupture of the mature schizont. Through screens using recombinant PfSUB1HA3, the authors isolated an inhibitor of this enzyme. Using this inhibitor, Yeoh et al. show that PfSUB1 activity is required for schizont rupture and erythrocyte reinvasion. They also show that schizont rupture and erythrocyte reinvasion is associated with the processing of an essential papain-like protease SERA5 (Miller et al., 2003) by PfSUB1 immediately prior to merozoite egress. Subsequent experiments confirmed SERA5 and other important SERA family members (4 and 6) that are expressed during schizogony as physiological substrates for PfSUB1.

This study provides the first major insight into the molecular mechanisms associated with merozoite egress from the infected erythrocyte. PfSUB1 activity is initially housed within the exoneme and is then released into the milieu of the parasitophorous vacuole where it digests and activates the papain-like proteases SERA4-6. The SERA proteases in turn play a role in the subsequent destruction of the parasitophorous vacuole and possibly the infected erythrocyte membrane itself thereby releasing the invading hordes of merozoites. These results support the "inside-out" model of membrane destruction during egress (Wickham et al., 2003). One relevant insight is that merozoite egress is a process controlled through the release of factors by the merozoite itself. Egress requires more cellular events than a simple activation signal originating within the lumen of the parasitophorous vacuole and is thus potentially more highly regulated. This is important because for infection to be maintained, egress requires that merozoite formation within the syncitium of the parasitophorous vacuole is a highly coordinated process (which it is) otherwise one or two merozoites racing ahead to maturity could trigger release of suboptimal numbers of fully mature parasites.

These are fascinating insights but like all new observations raise a host of additional questions some of which the authors also discuss. Dense granules should no longer be thought of as homogeneous, but are there only two classes—exonemes and "classic" dense granules—or are further subdivisions based on protein content and function possible for dense granules, rhoptries, and micronemes? How are proteins trafficked to the two different granule classes; do the signals overlap or are they completely different? Does SUB1 play a direct role in destroying parasitophorous vacuole and infected erythrocyte membranes, rather than merely activating the destroyers as is implied though not strictly proven here? Does SUB1 play a role in parasite egress at other points in the life cycle, for example liver-stage merozoite exit or sporozoite release from oocysts in the mosquito vector? Interestingly, another member of the SERA family, the ortholog of PfSERA8, is essential for sporozoite release from oocysts in the rodent malaria parasite, *Plasmodium berghei* (Aly and Matuschewski, 2005). Are there more substrate strings to the PfSUB1 bow, and are merozoite proteins themselves also targeted by PfSUB1 to perhaps promote the invasive ability of the merozoite? The primary processing agent for several merozoite surface proteins (such as MSP1, 3, 5, and 6) is unclear and may involve SUB1.

In terms of the value of SUB1 as a therapeutic target, it is worrying that the IC_{50} established for their recombinant PfSUB1 inhibitor MRT12113 soars when tested on parasites. In part, this decrease in efficacy might be due to accessibility, but it highlights the need for improved inhibitors. Successful screens may be informed by more information about the biology of SUB1 and its substrates. A multi-substrate protease, in principle and as shown with studies on HIV protease (reviewed in Louis et al., 2007), is an excellent drug target, because the likelihood of compensatory mutations in both the protease active site and the substrate cleavage site that might result in drug resistance is low. Clearly the tip of a large iceberg has been discovered, and we can anticipate an abundance of fundamental discoveries that will only strengthen the biologists' armory in the search for therapies against the pernicious diseases caused by *Plasmodium* and other apicomplexans.

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